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16. ABSTRACT The chapter explains the philosophy and usefulness of a phased approach in assessing potential environmental hazards of stationary source emissions, and gives examples of the experience and success of the approach to date. Recognition of the problems associated with the environmental release of wastes has resulted in environmental legislation, including the Clean Air and Clean Water Acts and their various amendments, and the Resource Conservation and Recovery Act (RCRA). This legislation enables federal and state governments to set and enforce emission standards which would control and manage the amounts of various pollutants released. To effectively implement these laws, it is necessary: (1) to identify waste stream constituents that are hazardous or toxic, (2) to determine acceptable release limits, and (3) to have available efficient, cost-effective control technology capable of meeting the established limits. The U.S. EPA has developed an environmental assessment program for obtaining such information for waste streams. The wastes from industrial and energy conversion processes constitute a major source of exposure for humans and other organisms. Large quantities of such wastes are released annually to the environment in gaseous, liquid, and solid forms. Exposure by organisms is by direct inhalation, ingestion, or absorption through food web transfer.

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Bioassay	Stationary Sources	06A, 06O
Toxicity	Environmental Assessment	06T
Wastes		14G
Emission		
Assessments		14B

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Development of New Bioassay Protocols

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The wastes from industrial and energy conversion processes constitute a major source of exposure for humans and other organisms. Large quantities of such wastes are released annually to the environment in gaseous, liquid and solid forms. Exposure by organisms is by direct inhalation, ingestion or absorption of the waste through food web transfer. Health and ecological effects from such exposure range from stimulation of growth in nutrient-limited organisms to irreversible changes, including death.

Recognition of the problems associated with the environmental release of wastes has resulted in environmental legislation, including the Clean Air and Clean Water Acts and their various amendments, and the Resource Conservation and Recovery Act (RCRA). This legislation enables federal and state governments to set and enforce emission standards which would control and manage the amounts of various pollutants

released. To effectively implement these laws it is necessary to (1) identify which constituents of waste streams are actually hazardous or toxic, (2) determine acceptable release limits, and (3) have available efficient, cost-effective control technology capable of meeting the established limits. The U.S. Environmental Protection Agency (EPA) has developed an environmental assessment program for obtaining such information about waste streams.

The EPA environmental assessment sampling and analysis program, developed at their Industrial Environmental Research Laboratory at Research Triangle Park, NC, has four components [1,2]:

1. evaluation of biological, chemical and physical characteristics of all waste streams;
2. identification of probable health and ecological effects of streams;
3. ranking of waste streams according to relative potential hazard; and
4. identification of appropriate pollution control technology programs.

The ultimate objective of this program is the development of a control technology program using both process engineering changes and addition of specific control devices to reduce emissions to the prescribed limits. The development of such a program requires a thorough understanding of the properties of the emission streams needing control, which in turn requires some procedure to identify the nature, extent and magnitude of the problem stream. Physical, chemical and biological studies are necessary to obtain the required understanding of the waste streams. Physical data (e.g., temperature, pressure or flowrates) are useful in determining rates of emission and in selecting appropriate procedures for control. Chemical information will identify the known pollutants as well as other constituents in a stream and provide data on concentrations which will aid in directing control activities. Biological data will permit an

overall assessment of a stream's toxicity, including that arising from known pollutants, compounds not yet identified as hazardous, and synergistic or antagonistic interactions among the various constituents.

Two approaches are possible for obtaining the necessary data to design a control technology program. The first is the direct approach. Each waste stream is subjected to a thorough sampling and biological, chemical and physical testing protocol. A relatively high degree of confidence is associated with the results of this approach. It is also expensive and time-consuming. A second approach, developed by the EPA [2] is called the phased approach. Studies are organized into three phases designed to reduce costs without compromising the accuracy or reliability of the resultant data. The initial phase (Level 1) acts as a screen using "grab" samples and simple tests (e.g., determination of chemical classes, static acute bioassays) to measure cost-effectively the basic chemical, physical and biological characteristics of each waste stream. Results are qualitative or, at best, semiquantitative and are used to rank the streams in order of relative hazard. The second phase (Level 2) involves more detailed sampling and analysis starting with the higher priority streams and working through the lesser priority streams as funds permit. Chemical tests will resolve the waste stream into its constituent compounds; biological tests will confirm and/or elaborate on level 1 results, providing more accurate data about the potential hazards. Finally, level 3 monitors specific pollutants of interest and the effects of various operating conditions on the streams. Chronic bioassays may be included in level 3, but levels 1 and 2 depend primarily on acute bioassays. The procedures for implementing the phased approach are under continual development and refinement. As of 1980, levels 1 and 2 sampling procedures and chemical analyses, and level 1 biological procedures were well along in development and refinement. Level 3 for sampling and chemical analyses and levels 2 and 3 for biological analyses were still under development. Because the phased approach is so reasonable, important and timely, the focus of the remainder of

this chapter will be on the level 1 biological testing protocol and its implementation in selected EPA environmental assessment programs.

LEVEL 1 BIOLOGICAL TESTING PROTOCOL

Development of the current (1980) level 1 biological testing protocol began in 1976 when EPA formed its Bioassay Subcommittee composed of selected health and ecological effects scientists from several EPA research and development laboratories. During this four-year period, the subcommittee has applied the principles of the phased approach to the development of level 1 biological analysis procedures [3], field tested the procedures via pilot studies, and revised and refined the procedures to solve problems revealed by the pilot studies and subsequent implementation. The following describes the procedures for this important protocol.

Level 1 Sample Collection and Preparation

Level 1 begins with the proper collection, transport and preparation of samples of the waste streams from a facility [4]. Care must be taken to get the best possible samples within the time and cost constraints imposed at level 1. These constraints dictate "grab" type samples taken at a single point in time during the operating cycle of the facility. All waste streams—gaseous, liquid and solid—must be sampled.

Sample Collection and Transport

The large-volume gaseous sample needed for one of the biological tests—the plant stress ethylene—is collected using a sampling probe inserted into an appropriate port in the stack. If the stream is under positive pressure, the sampling bags, made of Tedlar® (a relatively inert material), are filled with

the aid of a polypropylene regulating valve in the Teflon® sampling line to prevent bursting the bag. Negative pressure streams are sampled by placing an evacuated Tedlar sample bag connected to the sample line into a 55-gal fiberboard drum and evacuating the drum. As the negative pressure in the drum exceeds that of the waste stream, the bag will fill. These procedures are designed to prevent the gas stream from contacting anything but inert materials, thus reducing the possibility of contamination and preserving sample integrity to the best possible degree. A second, much smaller, sample of gaseous streams is taken using a source assessment sampling system (SASS), which removes suspended particles of various sizes using a series of cyclones and a filter and organics using a sorbent column [4]. The particulates and solvent samples are transported in amber glass, while large volume gas samples are shipped in the Tedlar bags protected by 55-gal fiberboard drums.

Liquid samples are collected in glass or linear polyethylene containers using a tap in the waste stream or a dipper or some other means for withdrawing the sample. Large volumes are required for the aquatic tests. Samples are cooled to 4°C for transport and storage prior to testing.

Simple grab samples using manual or auger (boring) collection techniques are suitable for solids. These samples are placed in amber glass or linear polyethylene containers for shipping and storage.

While the objective of level 1 sampling is to obtain representative waste stream samples, no attempt is made to collect them under either isokinetic or isothermal conditions nor to account for temporal variations in the process and its wastes.

Sample Preparation

There is a need for many of the samples to undergo preparation to make them acceptable for use in the biological tests (see Figure 1). For example, particulates and solids used in the health effects test needed to be respirable size (5 μm or less). Consequently, samples need to be sized to ensure the use

of 5 μm or smaller particles. If the quantity of small particles is insufficient, grinding may be required. The extraction of organics from the sorbent columns used to filter gaseous or liquid samples usually involves the use of methylene chloride, which is incompatible with the health effects tests. Solvent exchange using dimethyl sulfoxide (DMSO) to replace the methylene chloride is required. Solid samples to be tested via aquatic tests must undergo leachate preparation. A modified ASTM-A (American Society for Testing and Materials) aqueous leach procedure is used to generate sufficient quantities of leachate for aquatic testing. Other sample preparation procedures include filtering larger particles (greater than 2mm) from liquid samples and concentrating some dilute aqueous samples for use in the Ames and rodent tests to raise the concentration of constituents above each test's threshold limit.

Thus, all level 1 sample preparations are done under carefully controlled conditions and using specific equipment and materials to prevent contamination of the sample.

LEVEL 1 BIOLOGICAL TESTS

Several criteria were used in the development of the level 1 protocol. Paramount, of course, was the need to assess the health and ecological effects of a wide diversity of waste streams. Toxicity and mutagenicity (presumed carcinogenicity) are the key effects to be detected by the health tests. The use of both in vitro and in vivo test systems was also a criterion for the selection of health effects tests. Measurement of toxicity was the key to the ecological test selection. Also, the inclusion of different kinds of organisms (e.g., microorganisms, plants and animals) and different habitats (e.g., aquatic, terrestrial and marine) was deemed important. Other criteria included (1) a short testing period, no longer than two or three weeks, that resulted in acute as opposed to chronic or life-cycle tests and (2) low cost for each test; each was generally to cost less than $1500. Finally, each test incorporated into the protocol

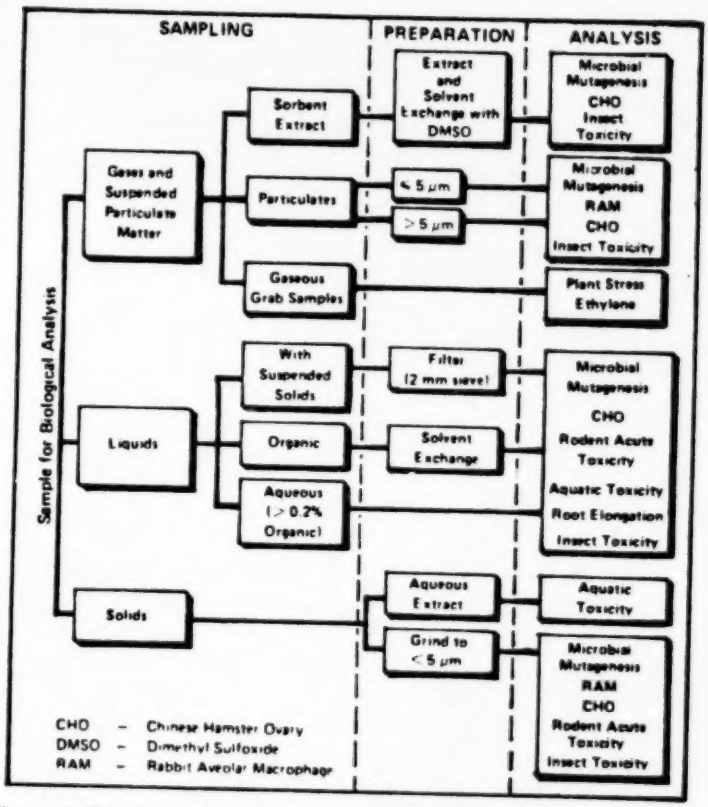

Figure 1. The level 1 biological testing protocol consists of a variety of organisms representing a variety of habitats. Organisms are exposed to chemical mixtures.

was to be acceptable by the scientific community as valid, reliable and accurate with a sufficiently large existing data base to facilitate the interpretation of the results. The application of these criteria resulted in the selection of the biological tests shown in Table I. The application of these tests to specific kinds of environmental samples is shown in Figure 1 [3].

All of the level 1 biological tests follow the same basic procedure. Test organisms are separated into groups, and each

Table I. 1980 EPA Level 1 Recommended Health and Ecological Effects Tests

Health Effects	Ecological Effects
In vitro	Aquatic[a]
Ames microbial mutagenesis assay (Ames)	Algal toxicity test
Rabbit alveolar macrophage cytotoxicity assay (RAM)	Static acute invertebrate toxicity test
Chinese hamster ovary cell clonal toxicity assay (CHO)	Static acute fish toxicity test
In vivo	Terrestrial
Rodent acute toxicity test	Plant stress ethylene test
	Seed germination–root elongation test
	Insect toxicity test

[a]Both marine and freshwater species are used in the aquatic effects tests.

group is exposed to one concentration of the test sample. Concentrations are fixed for each test and range from the maximum applicable doses down to some small fraction of that dose (e.g., 0.1 or 1%). There are usually five or six concentrations per test plus the negative control (zero concentration). Several tests use the results of this type of exposure as a rangefinding test and follow up with a definitive test with four or five concentrations bracketing the projected concentration (based on the rangefinding results) which produces the prescribed test endpoint. All tests have acute short-term exposures and use lethality or a similar measure (e.g., reduced growth) as the endpoint.

Health Effects Tests

The purpose of the health effects tests is to obtain data that will permit predictions of the potential hazard of complex waste streams to humans. The in vitro tests are designed to measure mutagenicity and toxicity at the cellular level. For example, the Ames microbial mutagenesis assay employs a mutant strain of the bacterium, *Salmonella*, as the test species and measures sample-induced mutation back to the normal

form [5–7]. Cytotoxicity is also measured during this test, and results are expressed as the concentration at which the number of bacterial colonies is half that of the negative control (LC_{50}). While not a mammalian cell assay, it has been observed that many chemicals found to be carcinogenic in long-term mammalian studies also elicit a positive Ames test result. Thus, a positive Ames test identifies the potential of the sample being carcinogenic to mammals, including humans.

Cytotoxicity tests use mammalian cells to measure the toxic effects of samples [8–11]. The RAM test is used on respirable particulates (5 μm or less) and solid samples ground to respirable size. The CHO assay is used on liquid and solid samples. Both cell types are phagocytic and will ingest small particles. centration at which the responses of the experimental cultures centration at which the response of the experimental cultures reach one-half that of the controls. Cell viability and ATP content are the typical responses or endpoints measured. These results are an indicator of the response which might be observed in mammalian tissues of whole animals exposed to the sample. However, the in vitro nature of these tests greatly tempers any quantitative extrapolation to the whole animal.

Recognizing the difficulties in such extrapolations, an in vivo animal rodent acute toxicity test was included as part of level 1 [12–14]. Laboratory mice are used as the test organisms. Test endpoints are lethality (LD_{50}) plus pharmacological toxicity observations for nonlethal responses.

Ecological Effects Tests

The ecological effects tests are included in level 1 to provide an indication of the effects complex waste streams could have on organisms other than man. Species living in both aquatic and terrestrial habitats are tested since wastes are released to both the water and land. Finally, organisms are used that represent different levels of complexity ranging from unicells to higher plants and vertebrates to determine effects of

wastes on different kinds of metabolic pathways and physiologies.

The aquatic ecological effect tests use algal, invertebrate and vertebrate species from both freshwater and marine habitats in acute toxicity testing. *Skeletonema costatum* and *Selenastrum capricornutum* are the marine and freshwater algal species, respectively. Invertebrates include *Daphnia magna* for freshwater testing and *Mysidopsis bahia* for marine testing. The marine vertebrate is the juvenile sheepshead minnow, *Cyprinodon variegatus*, while the fathead minnow, *Pimephales promelas*, is the freshwater vertebrate of choice. The vertebrates and invertebrates are tested using static acute procedures [15, 16]. The LC_{50} is the endpoint for these tests. The algal test protocol monitors changes in growth rate as a measure of toxicity [17]. The test endpoint is the effective concentration of sample which reduces algal growth by 50% as compared to the controls (EC_{50}). A second endpoint, the concentration at which increased growth is observed (stimulation concentration, SC), is determined for samples which may stimulate algal growth by supplying additional quantities of a normally limiting nutrient.

The level 1 terrestrial tests and test protocols had not as of 1980 been made final. The originally proposed tests [3] were unable to meet all the level 1 criteria as revealed by the pilot studies conducted in 1977. There have been subsequent modifications and refinements to the terrestrial protocol, but the ability of the resultant protocol to meet level 1 needs has not been fully validated. The current protocol includes the plant stress ethylene, seed germination–root elongation, and insect toxicity tests [18]. The plant stress ethylene test uses increased ethylene production as its endpoint [3,19]. Many plants show changes in ethylene production in response to stress; this response can be very sensitive and more replicable than the more traditional visual damage parameters often used to measure plant stress. The bushbean (*Phaseolus vulgaris*) is the test species. The test is used primarily on gaseous samples. Modifications to the basic test protocol are underway.

The seed germination–root elongation test, a recent addition

to the level 1 ecological effects protocol, measures the effect of liquid or leachates from solid wastes on the germination and growth of several species of plants [20,21]. Test endpoints include percent germination and root length and results are expressed as the effective concentration required to reduce either measure by 50% (EC_{50}). The procedures for this test were still being refined and validated as of late 1980.

The final terrestrial test is the insect toxicity bioassay using *Drosophila melanogaster*. This test, also a new addition to level 1, is still to be validated. It involves acute exposures to various concentrations of liquid or solid samples with the test endpoint being lethality expressed as LD_{50}.

Quality Control and Quality Assurance (QC/QA)

The EPA is in the process of implementing QC/QA procedures to ensure the reliability and replicability of the level 1 biological test results. Such procedures range from proper labeling of a new sample through the use of a standard format for reporting test results. Furthermore, controls will be required for each test, a minimum being the negative (zero concentration) type of control with the addition of positive controls and solvent controls for certain tests. Standard test protocols must be written and available to the technical staff performing the bioassay. Any deviations from these standard protocols must be authorized by the EPA Project Officer and fully reported. Instruments must be calibrated regularly. There will be a strong emphasis on written documentation, and reports will be reviewed objectively for accuracy and consistency. Eventually all level 1 bioassays will be subject to the EPA Good Laboratory Practice (GLP) regulations.

Data Reporting and Interpretation

There are three levels of data reporting. The first is the raw test data. The actual laboratory data sheets are required show-

ing data entries just as they were recorded in the lab. The second level reporting provides the toxicity or mutagenicity calculation (e.g., LC_{50} and EC_{50}) for each test. The final level is a nonnumerical evaluation of toxicity useful for a quick overview of the bioassay results and, when used with the level 1 chemistry data, permits the relative ranking of waste streams. Conversions from LC/EC data to these relative rankings are given (Table II).

APPLICATION OF LEVEL 1 BIOASSAYS TO FLUIDIZED BED COMBUSTION SAMPLES

One major EPA environmental assessment program where the phased approach has been applied, was a study of the fluidized bed combustion (FBC) process. FBC offers a feasible alternative for burning high-sulfur coal for steam power generation with air emissions of appreciably reduced environmental impact. When a sorbent material such as limestone or dolomite is fed with the coal into a combustion chamber maintained near 815°C, the sorbent reacts with the sulfur to produce a sulfate which can be removed and disposed with the solid ash materials. The resulting ash materials are discarded in a form more biologically available than ash from other combustion facilities. This characteristic justifies increased attention to the biological significance of the solid residues discharged as suspended particles in the flue gas, the particulate solids rejected from the system in bulk form and the leachates which might result from ground disposal of these particulate solids.

This EPA program studied, among others, a small demonstration pressurized FBC facility that burned coal with dolomite under pressure to generate heat for the production of steam plus several waste streams: (1) coarse particulates, (2) fine particulates, (3) gas, (4) second cyclone filter catch, and (5) bed reject material. Simple grab samples were taken of the coal, dolomite and waste streams 4 and 5 during the normal operation of the FBC facility. The SASS was used

Table II. Evaluation Criteria for Level 1 Biological Tests Used To Develop a Relative Ranking Based on Toxicity of Waste Streams

Biological Test	Units	Relative Sample Toxicity			
		High	Moderate	Low	Not Detectable
Ames assay					
Solid Samples	Lowest concentration[a] (mg/plate)	<0.5	0.05–0.5	0.5–5	>5
Liquid Samples	Lowest concentration[a] (μl/plate)	<2	2–20	20–200	>200
RAM and CHO					
Solid Samples	EC_{50} (μg/ml)	<10	10–100	100–1000	>1000
Liquid Samples	EC_{50} (μl/ml)	<6	6–60	60–600	>600
Rodent test	LD_{50} (g or ml/kg)	<0.1	0.1–1	1–10	>10
Aquatic tests	EC_{50}/LC_{50} (percent sample)	<20	20–75	75–100	>100
Plant stress ethylene	Lowest mixing time[a] (min)	1	2	7	>7
Seed germination–Root Elongation	EC_{50} (percent sample)	<0.01	0.01–1	1–100	>100
Insect toxicity					
Solid Samples	LD_{50} (mg/ml)	<0.5	0.5–5	5–50	>50
Liquid Samples	LD_{50}(μl/ml)	<5	5–50	50–500	>500

[a]Lowest concentration (Ames) or time (stress ethylene) giving a positive response.

to collect samples from waste streams 1 to 3. The samples were contained and transported according to level 1 procedures to the laboratory where level 1 biological and chemical analyses were performed.

The selection of the bioassays for use on the collected samples followed the Level 1 test protocol as described in Figure 1. Leachates were made of the large quantity solid streams—second cyclone catch, bed reject, coal and dolomite—for use in the aquatic tests. Since these solid streams, on exposure to or disposal in the environment, had the potential of leaching into both freshwater and marine habitats, both marine and freshwater species were used in the aquatic tests.

Health effects results from the FBC waste streams were observed only from the particulate samples when low levels of mutagenicity were found (Table III). The fine particulates also elicited a cytotoxic response. The rodent test detected no toxicity in any of the samples. In the ecological tests, coal leachate was toxic in all the aquatic tests (Table III). The bed reject leachate caused a toxic response in the freshwater, but not in the marine tests. Some toxicity was observed with second cyclone leachate (*Daphnia*) and the dolomite leachate (marine algae and invertebrates). No toxicity was observed in the plant stress ethylene test.

Level 1 chemical analyses were conducted on most of the FBC samples (dolomite and coal feedstocks excluded). Results were expressed as the ions or chemical classes which exceeded the discharge multimedia environmental goal (DMEG) value. This value is defined as the maximum concentration of a chemical believed to be safe for short term exposure to selected organisms [22]. DMEG values are derived primarily from laboratory animal studies. Using the chemical data expressed as those chemical constituents which exceed the DMEG value, comparison of the biological and chemical results can be made (Table IV). In general, there is positive correlation between the trends in biological toxicity and the chemical analyses with the gas sample being an exception. The lack of positive response in the plant stress ethylene test, even though several

Table III. Level 1 Bioassay Results for FBC Samples Including Toxicity Data and Relative Toxicity Evaluations[a]

Bioassay	Test Endpoint	Sample								
		Coarse Particulates	Fine Particulates	Gas	Second Cyclone	Second Cyclone Leachate	Bed Reject	Bed Reject Leachate	Coal Leachate	Dolomite Leachate
Ames	+/−	+ (L)	+(L)							
RAM	EC_{50} (μg or μl/ml)	>1000 (ND)	596 (L)		>1000 (ND)	>600 (ND)	>1000 (ND)	>600 (ND)		
Rodent	LD_{50} (g or ml/kg)				>10 (ND)	210 (ND)	710 (ND)	710 (ND)		
Freshwater										
Algae	EC_{50} (%)									
Daphnia	LC_{50} (%)					>100 (ND)		45 (M)	19 (H)	>100 (ND)
Fish	LC_{50} (%)					92 (L)		41 (M)	71 (M)	>100 (ND)
Marine						>100 (ND)		25 (M)	92 (L)	>100 (ND)
Algae	EC_{50} (%)									
Invertebrate	LC_{50} (%)					>100 (ND)		>100 (ND)	38 (M)	24 (M)
Fish	LC_{50} (%)					73 (M)		>100 (ND)	66 (M)	75 (M)
Ethylene Stress (Plant)	Exposure (min)			>7 (ND)		>100 (ND)		>100 (ND)	72 (M)	>100 (ND)

[a]H = high toxicity, M = moderate toxicity, L = low toxicity, ND = not detected (follows ranking scheme in Table II).

toxic constituents were present in the gas, may result in the insensitivity of the plant test (DMEG values are based primarily on animal data) to the chemicals present. Other considerations are possible antagonistic interactions among the constituents reducing the net toxicity of the sample, or deterioration of the sample during the elapsed time between collection and testing with toxicants adsorbing, precipitating, reacting or permeating through the walls of the sample container. The toxicity observed in the bed reject appears anomalous to the other samples in that it showed significant toxicity to all freshwater species and none to marine. This effect likely resulted from the high salt concentration which created osmotic imbalances in the freshwater species but had no effect on marine species tolerant of relatively high salt content. The FBC bed reject, coal and dolomite leachates, and particulate samples would all have high priority for level 2 testing. The gas and second cyclone samples would have lower priority for additional study.

LEVELS 2 AND 3 BIOLOGICAL TESTING

The development and refinement of level 1 bioassay protocols are quite advanced and they are now routinely implemented in EPA environmental assessment programs. The levels 2 and 3 bioassay protocols are still under development, both in concept and specific details. The basic objective of level 2 is to verify and elaborate on the level 1 results and to locate the probable cause of toxicity. For high-priority hazardous streams this will likely involve sample fractionation and subsequent testing. The fractionation procedure will likely involve separation of the stream into organic and inorganic components which would be tested. Further fractionation would be done on the toxic fraction(s): organics into acid, base, and neutral components and inorganics into cations and anions. These subfractions would also be tested. The testing of the fractions may use health and ecological bioassays simi-

lar to those employed at level 1 with additions such as increased number of replicates or test organisms which will improve the accuracy and reliability of the tests or a change in test species. New bioassays may be added such as teratogenicity, short-term chronic and life-cycle testing protocols. The objective of this scheme is to identify which component(s) of the waste system is toxic and to obtain information on the nature and magnitude of that toxicity.

Table IV. Comparison of Level 1 Biological and Chemical Results for Fluidized Bed Combustion Samples

Sample	Biological Results	Chemical Results
Coarse & Fine Particulates	Low mutagenicity, low cytotoxicity	Al,As,Be,Ca,Cd,Fe,Hg,K, Sb,Se,Si, aliphatic hydrocarbons >DMEG
Gas	No detectable toxicity	As,Cl,H_2SO_4,NO_x,Se,SO_2, >DMEG
Second Cyclone	Low to moderate toxicity only in aquatic invertebrates	Numerous elements >DMEG
Bed Reject	Moderate freshwater toxicity	Fe,Ni >DMEG, high salt concentration
Coal	Toxic in aquatic tests	No data
Dolomite	Moderate marine toxicity	No data

Level 3 will focus on process variations which result in changes to the chemistry of the waste streams. Flow-through testing may be employed where the concentrations to which the test organisms are exposed vary as the waste stream varies. Long-term chronic and subacute (nonlethal) testing may also be used to determine more subtle effects on the test species. This work will support the control technology development and implementation efforts.

Development of the levels 2 and 3 biological test protocol has not reached the pilot study stage. Considerable work remains to bring these activities up to the current stage of development for level 1. Importantly, results of level 1 are being

applied in a variety of situations, including the textile manufacturing industry shown by Rawlings [23].

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